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Short communication

Prevalence of *Sarcocystis neurona* and *Neospora* spp. infection in horses from Brazil based on presence of serum antibodies to parasite surface antigen

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Abstract

Sera from 961 horses from Brazil were tested for antibodies against the major surface antigens SnSAG4 and NhSAG1 to determine the seroprevalence of *Sarcocystis neurona* and *Neospora hughesi*, respectively. Antibodies against SnSAG4 were detected in 669 (69.6%) of the horses, while antibodies against NhSAG1 were detected in only 24 (2.5%) of the horses. These serologic results suggest that there is a high concentration of *S. neurona* in the environment of Brazil, which results in marked exposure of horses to this parasite. Additionally, the data further confirm that infection with *Neospora* spp. is relatively uncommon in horses.

Keywords: *Sarcocystis neurona*; *Neospora* spp.; Seroprevalence; Horses; ELISA; Brazil

1. Introduction

Equine protozoal myeloencephalitis (EPM) is a debilitating neurologic disease of horses that is caused by the protozoan parasites *Sarcocystis neurona* and *Neospora hughesi* (Dubey et al., 2001c; MacKay et al.,

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2000). Horses are infected with *S. neurona* through ingestion of sporocysts passed in the feces of opossums, which are the definitive host for *S. neurona* (Fenger et al., 1995). Extensive seroprevalence studies conducted in a variety of geographic locations of the United States have demonstrated that infection of horses with *S. neurona* is common (Bentz et al., 1997; Blythe et al., 1997; Saville et al., 1997; Tillotson et al., 1999), with reported seroprevalence ranging from approximately 34% in Colorado to 54% in Ohio. The definitive host for *N. hughesi* has not yet been determined, so it remains unknown how horses are exposed to this parasite. It is also unclear whether horses can be infected with the related species *Neospora caninum*. However, a large sero-survey recently indicated that *Neospora* spp. infection is relatively uncommon in horses from North America (Hoane et al., 2005b).

In addition to the North American opossum *Didelphis virginiana* (Dubey and Lindsay, 1998), the South American opossum *Didelphis albiventris* has been shown to be capable of transmitting *S. neurona* (Dubey et al., 2001b). Consistent with the presence of a competent definitive host, *S. neurona* infection of horses has been observed in both Brazil (Dubey et al., 1999a) and Argentina (Dubey et al., 1999b). In these prior studies, 36% of the horses tested in Brazil and 35% of the horses in Argentina had antibodies against *S. neurona*, based on western blot analyses. Antibodies against *Neospora* spp. were not detected in any of the horses examined with the *Neospora* agglutination test. The set of serum samples from Brazil was obtained from 101 healthy Thoroughbred horses (Dubey et al., 1999a), and the samples from Argentina were obtained from 76 draft type horses belonging to the Argentinean army (Dubey et al., 1999b).

In the present survey, we have investigated the presence of antibodies against *S. neurona* and *Neospora* spp. surface antigens in a large number of horses from Brazil. Importantly, many of the horses tested herein were older animals that may have been in poorer condition and maintained with less favorable husbandry than the horses in the previous South American serologic surveys.

2. Materials and methods

Blood was obtained from 961 horses from 10 different states in Brazil (São Paulo and Minas Gerais, southeast region; Paraná, Santa Catarina and Rio Grande do Sul, south region; Bahia, northeast region; Rondônia, north region; and Mato Grosso, Mato Grosso de Sul and Goiás, middle west region). A majority of the samples were from older horses (greater than 10 years) that were culled and sent to a slaughter house. A portion of the samples were obtained from farms where the horses were used to work cattle. Eleven samples were from horses sent to slaughter, but with no history providing their origin. Serum was separated, frozen at −20 °C, and sent to the USA for antibody detection.

To determine *S. neurona* seroprevalence, antibodies against the parasite surface antigen SnSAG4 were detected using an enzyme-linked immunosorbent assay (ELISA) that has been described previously (Hoane et al., 2005a). A conservative cut-off of 25% positivity (PP) was used to avoid overestimating the *S. neurona* seroprevalence. At this cut-off, the SnSAG4 ELISA previously exhibited 91% sensitivity and 86% specificity relative to the standard *S. neurona* western blot (Hoane et al., 2005a).

For determination of *Neospora* spp. seroprevalence, antibodies against the parasite major surface antigen NhSAG1 were detected using a previously described ELISA (Hoane et al., 2005b). A PP of 20 was used for the positive–negative cut-off. This cut-off has been shown to give a slight overestimation of the *Neospora* seroprevalence based on comparison to western blot analysis (Hoane et al., 2005b).

3. Results and discussion

A total of 669 (69.6%) of the 961 equine serum samples tested positive for antibodies against *S. neurona* surface antigen SnSAG4 (Table 1). The highest seroprevalence was observed in the state of Minas Gerais (90%; $n = 10$) and in samples collected from unknown regions (90.9%; $n = 11$). However, the number of sera in these two sample subsets was relatively small. For states where a substantial number of samples were collected, seroprevalence of 84.4% to *S. neurona* was observed in the 192 horses tested from Rondônia. This state has a tropical, humid climate and is located in the northwestern portion of Brazil. Rondônia is heavily forested with savannahs that are utilized for agriculture. The lowest seroprevalence to

Table 1
Seroprevalence of *S. neurona* and *Neospora* spp. in Brazil horses based on detection of antibodies to the parasite surface antigen SnSAG4 and NhSAG1, respectively

State, region	Total tested	*Sarcocystis neurona* positive (%)	*Neospora* spp. positive (%)
São Paulo, SE	513	348 (67.8)	15 (2.9)
Minas Gerais, SE	10	9 (90.0)	0 (0)
Santa Catarina, S	24	11 (45.8)	0 (0)
Rio Grande do Sul, S	2	1 (50.0)	0 (0)
Paraná, S	146	88 (60.3)	5 (3.4)
Mato Grosso, CW	28	15 (53.6)	0 (0)
Mato Grosso do Sul, CW	11	9 (81.8)	0 (0)
Goiás, CW	15	9 (60.0)	0 (0)
Rondônia, N	192	162 (84.4)	3 (1.6)
Bahia, NE	9	7 (77.8)	1 (11.1)
Unknown	11	10 (90.9)	0 (0)
Total	961	669 (69.6)	24 (2.5)

SE: southeast region; S: south region; CW: central west region; N: north region; NE: northeast region.

S. neurona was observed in the 24 horses tested from Santa Catarina. Santa Catarina is a small state with a fairly temperate climate and is located on the southern coast of Brazil.

The seroprevalence of *Neospora* spp. in the 961 horses was found to be only 2.5%, based on detection of antibodies to the parasite major surface antigen NhSAG1 (Table 1). The state of Bahia had the highest seroprevalence to *Neospora* spp., 11.1%, but only nine horses were tested from this region. Five (3.4%) of the 146 horses tested from Paraná had antibodies against NhSAG1 (Table 1).

The seroprevalence results obtained in this study suggest that the tested horses from Brazil had substantial exposure to *S. neurona* sporocysts, since approximately 70% of the 961 equine serum samples were found by ELISA to be seropositive to *S. neurona*. This is approximately double the seroprevalence observed in previous studies conducted in South America. Prior surveys of horses in Brazil (Dubey et al., 1999a) and Argentina (Dubey et al., 1999b) had found 36 and 35% of samples were seropositive to *S. neurona*, respectively. The discrepancy in seroprevalence is likely due to a fundamental difference between the horses tested in the current study and those examined previously. Specifically, the samples collected in Brazil were from healthy thoroughbreds; 70 samples were collected at the Jockey Club in São Paulo, 15 samples were from a training center in Rio de Janeiro, and 16 samples were from a farm in the state Rio Grande do Sul (Dubey et al., 1999a). All 76 horses tested from Argentina were from a single location and were owned by the Argentinean army (Dubey et al., 1999b). Consequently, it is likely that the horses tested in these previous studies were younger and healthier and had greater care than the horses surveyed in the present study. Indeed, many of the animals tested in the present study were older than 10 years, and some were culled horses that had been sent to slaughter houses. It is probable that unfavorable management practices contributed to the exposure of these horses to *S. neurona*, as suggested previously (Saville et al., 2000), but the age and condition of the horses also certainly influenced the results presented herein.

In general, the level of *S. neurona* infection observed in this study in South American horses is higher than what has been found in horses in North America. Initial studies examining exposure of horses to *S. neurona* in the United States found that seroprevalence was approximately 45–50% (Bentz et al., 1997; Blythe et al., 1997; Saville et al., 1997; Tillotson et al., 1999). Subsequent surveys have found 60% seroprevalence in 1121 horses in Michigan (Rossano et al., 2001) and a significantly higher 89% seropositive rate in 798 horses in Oklahoma (Bentz et al., 2003). Much lower *S. neurona* prevalence has been found in the Rocky Mountain region of North America, ranging from 34% in northern Colorado (Tillotson et al., 1999) to non-existent in Montana

(Vardeleon et al., 2001) and western Canada (Dubey et al., 2003). The variation in seroprevalence seen in different regions of North America has typically been attributed to climate and the density of competent hosts for *S. neurona*, in particular its definitive host, the opossum.

Given the level of *S. neurona* seroprevalence observed in this study, it is apparent that Brazil harbors a large presence of suitable definitive and intermediate hosts for this parasite. Furthermore, infection of these hosts with *S. neurona* must be relatively common. The white-eared opossum (*D. albiventris*) has been shown to be a competent definitive host for *S. neurona* (Dubey et al., 2001b), and it is likely that the black-eared opossum (*Didelphis marsupialis*) can similarly serve as a definitive host. The intermediate host(s) in South America has not been determined. However, Brazil has several species from the family Procyonidae (raccoons), which *S. neurona* may infect. Additionally, there are a variety of Edentates in Brazil, which are toothless animals that primarily eat ants and are therefore prone to ingesting coccidian oocysts/sporocysts. This group of animals includes sloths, anteaters, and armadillos, one species of which has been shown to be a North American intermediate host for *S. neurona* (Cheadle et al., 2001).

In contrast to the high *S. neurona* seroprevalence, only 2.5% of the horses tested in the current study had antibodies against the NhSAG1 major surface antigen of *N. hughesi*. These results are comparable to the 3.4% *Neospora* spp. seroprevalence found using the NhSAG1 ELISA in a recent survey of 1917 horses from North America (Hoane et al., 2005b). In two prior surveys in South America, detectable antibodies against *Neospora* spp. were not found in any of the horses that were tested (Dubey et al., 1999a, 1999b). Collectively, these studies indicate that horses in both North and South America are not commonly infected with *Neospora* spp. While it is clear that horses can be infected by *N. hughesi* (Cheadle et al., 1999; Dubey et al., 2001a; Marsh et al., 1998), it is possible that infection of horse may be limited to this single species since it remains unclear whether *N. caninum* infection occurs in horses. Further investigations to determine in better detail the biology and life cycle of both *N. hughesi* and *N. caninum* will help to clarify this issue.

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